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Research Article

Synthesis and *In-Vitro* Antimicrobial screening of some new Pyrazolone based Schiff Bases

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ABSTRACT

Pyrazolone Schiff base derivatives are an important class of heterocyclic compounds that occur in many drugs and synthetic products. It has a particular value due to their broad spectrum of biological activities and their utility as synthetic tools in the design of various bioactive molecules. It is also exhibited analgesic, antipyretic and anti-inflammatory activity. In the present study, a series of (5*E*)-3-methyl-*N*-substitutedphenyl-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (**5a-g**) were synthesized by the reaction of pyrazolone and sulfanilamide in ethanol. The synthesized compounds were characterized on the basis of IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. All synthesized Compounds were screened for antimicrobial activities using disc diffusion technique against two bacterial pathogens viz *Staphylococcus aureus* and *Pseudomonas aeruginosa* and two fungal pathogens namely *Candida albicanes, Aspergillus niger*. Chloramphenicol and Fluconazole were used as standard drug respectively. The compounds exhibit moderate activity.

Keywords: Pyrazolones; Sulfanilamide; Antimicrobial activity; Disc diffusion technique.

1. INTRODUCTION

Schiff bases are an important class of compounds in organic chemistry exhibiting in many cases biological and pharmacological activities[1-5]. Several Schiff bases, some containing different heterocyclic moieties, have been reported to exhibit anticancer[6], antibacterial[7], antifungal[8,9], herbicidal[10], cytotoxic[11], anticonvulsant[12], and antiproliferative[13] activities. Pyrazolone is a widely used precursor to a variety of compounds, documented well for their numerous applications such as products and intermediates in analytical, agricultural, biological, and pharmaceutical chemistry[14-17]. Antipyrine was the first pyrazolones derivative for clinical use and was synthesized in 1883 [18,19]. Pyrazolones have gained importance as drug substances in pharmaceutical industry in view of their biological importance. For instance, the pyrazolones, viz. phenazone, propyphenazone, ampyrone and metamizole are useful antipyretic and analgesic drugs[20]. In addition, pyrazolones possess antimicrobial, antifungal[21], antimycobacterial[22,23], antibacterial[24], anti-inflammatory[25], antitumor[26], gastric secretion stimulatory[27], antidepressant[28] and antifilarial activities[29].

The sulfonamides or sulfa drugs are synthetic antimicrobial agents with a wide spectrum encompassing most gram-positive and many gram-negative organisms. These drugs were the first efficient treatment to be employed systematically for the prevention and cure of bacterial infections. Many reports exist on a ligational behaviour of pyrazolone based Schiff compounds. But, to the best of our knowledge, no reports exist on their biological evaluation. The presence of fragment –N,CH–R in Schiff bases is known for its biological activity[30,31]. Many reports exist on structure–activity relationship of the class of these compounds[32,33]. Thus, it seems worthwhile to continue to investigate in this area. In the present work, we report synthesis and antimicrobial activities of some pyrazolone based Schiff

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bases derived from a condensation reaction of pyrazolones with sulfanilamide.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents used

All melting points were determined in open capillaries and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck) with acetone/n-hexane (1:3). The IR spectra (in KBr pellets) were recorded on a spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded ($CDCl_3$) on a Bruker DRX 300 (300MHz, FT NMR) and varian (300MHz) spectrometer. The Mass spectra were recorded on a Jeol –SX 102 spectrometer. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. Commercial grade solvents and reagents were used without further purification.

2.2 General procedure for the synthesis of (5*E*)-3-methyl-*N*-phenyl-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (Schiff bases) (5a-g)

The synthetic route has been highlighted in **Scheme 1**. For the synthesis of the titled compounds, 3-methyl-*N*-(substituted phenyl)-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbothioamide (3a-g) was prepared by reacting phenyl thiosemicarbazide with ethylacetoacetate in the presence of DMF. The reaction of equimolar quantities of pyrazolone with sulfanilamide were refluxed in the ethanol for 8 hrs resulted in the target compound (**5a-g**).

2.3 Preparation of 3-methyl-*N*-(substituted phenyl)-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbothioamide (3a-g)

Take intimate mixture of phenyl thiosemicarbazide (0.01 mole, 1.67 gm), ethylacetoacetate (0.01 mole, 1.28 mL) and dimethyl formamide (DMF) 25 mL were taken in a round bottom flask fitted with air condenser and refluxed for 10 hrs. at 80-90^{o}C. Now mixture was melted and obtained transparent yellow colour. The progress of the reaction was checked by TLC. Refluxing was continued till the reaction was completed. After completation of reaction mixture was allowed to stand overnight, next day excess of solvent was distilled off and the resultant residue was poured on crushed ice with few drops of H_2SO_4. The solid precipitated were filtered and recrystallized with ethanol. Yield= 70%, M.P. = 130^{o}C

2.4 Synthesis of Schiff bases (5a-g)

Take intimate mixture of pyrazolones (0.01 mole), sulfanilamide (0.01 mole) and ethanol 25 mL were taken in a round bottom flask fitted with air condenser and refluxed for 8 hrs. at 80-90^{o}C. The progress of the reaction was checked by TLC. Refluxing was continued till the reaction was completed. After completation of reaction mixture was allowed to stand overnight, next day excess of solvent was distilled off and the resultant residue was poured on crushed ice with few drops of H_2SO_4. The solid precipitated were filtered and recrystallized with ethanol. Yield= 70%, M.P. = 130^{o}C

2.5 Yield, Melting point (M.P.) and Spectral data

(5*E*)-3-methyl-*N*-phenyl-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5a)

Yield-68%, M.P.-120^{o}C, IR(KBr, ν_{max} cm^{-1}): 3250 (N-H-Str), 1693 (C=O), 1425 (C-N-Str), 1159 (C=S), 1610(C=N); ^{1}HNMR=(300MHz,DMSO)δ=1.1(3H,s), 2.1(2H,s), 2.6(2H,S), 4.5(1H,s), 6.49(2H,t), 6.69(1H,t) 7.05(2H,m), 7.5(2H,t), 8.0(2H,t)ppm; ^{13}C NMR: δ 186.0, 173.2, 156.9, 152.2, 137.8, 140.4, 128.8, 126.8, 125.9, 124.7,122.3, 36.2, 20.6; MS(EI) m/z=387.08[M]$^{+}$, Anal. calcd. For $C_{17}H_{17}N_5O_2S_2$: C,56.63; H,4.75; N,18.01; Found: C,56.43; H, 4.64; N, 18.07.

(5*E*)-3-methyl-*N*-(2-methylphenyl)-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5b)

Yield-70%, M.P.-215^{o}C, IR(KBr, ν_{max} cm^{-1}): 3178(N-H-Str), 1699 (C=O), 1452 (C-N-Str), 1157 (C=S), 1602 (C=N); ^{1}HNMR=(300MHz,DMSO)δ=1.02(3H,s), 2.1(2H,s), 2.3(2H,s), 2.39(3H,s), 4.3(1H,s), 6.44(1H,d), 6.60(1H,t), 6.89(2H,dd), 7.5(2H,t), 8.0(2H,t)ppm; ^{13}C NMR: δ 186.0, 164.2, 155.8, 152.2, 141.1, 137.8, 134.6, 129.8, 126.8, 125.5, 124.6, 35.8, 20.9, 12.9; MS(EI) m/z=401.10[M]$^{+}$, Anal. Calcd. for $C_{18}H_{19}N_5O_2S_2$: C,58.28; H,5.30; N,16.99; Found: C,58.03; H, 4.94; N, 16.27.

(5*E*)-3-methyl-*N*-(3-methylphenyl)-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5c)

Yield-67%, M.P.-159^{o}C, IR(KBr, ν_{max} cm^{-1}): 3265 (N-H-Str), 1672 (C=O), 1476 (C-N-Str), 1166 (C=S), 1620 (C=N);

^{1}HNMR=(300MHz,DMSO)δ=1.09(3H,s), 2.1(2H,s), 2.22(2H,s), 2.41(3H,s), 4.20(1H,s), 6.37(2H,d), 6.52(1H,d), 6.91(1H,dd), 7.5(2H,t), 8.0(2H,t)ppm, ^{13}C NMR: δ 186.9, 165.2, 156.1, 153.2, 139.7, 138.1, 128.9, 126.5, 126.9, 125.8, 122.8, 30.4, 21.1, 16.4; MS(EI) m/z=401.10[M]$^+$, Anal. calcd. for $C_{18}H_{19}N_5O_2S_2$: C,57.28; H,4.90; N,17.99; Found: C,57.03; H, 4.94; N, 17.27.

(5*E*)-3-methyl-*N*-(4-methylphenyl)-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5d)

Yield-71%, M.P.-180^{o}C, IR(KBr, ν_{max} cm^{-1}): 3256 (N-H-Str), 1668 (C=O), 1482 (C-N-Str), 1141 (C=S), 1605 (C=N); ^{1}HNMR=(300MHz,DMSO)δ=1.20(3H,s), 2.1(2H,s), 2.32(2H,s), 2.61(3H,s), 4.10(1H,s), 6.54(2H,t), 6.85(2H,t), 7.5(2H,t), 8.0(2H,t)ppm; ^{13}C NMR: δ 186.3, 165.5, 155.7, 152.2, 137.8, 136.9, 134.7,133.7, 130.5, 126.1, 122.3, 30.4, 21.3, 16.3; MS(EI) m/z=401.10[M]$^+$, Anal. calcd. for $C_{18}H_{19}N_5O_2S_2$: C,58.20; H,5.50; N,16.89; Found: C,58.13; H, 4.99; N, 16.77.

(5*E*)-*N*-(2-methoxyphenyl)-3-methyl-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5e)

Yield-65%, M.P.-155^{o}C, IR(KBr, ν_{max} cm^{-1}): 3300 (N-H-Str), 1686 (C=O), 1463 (C-N-Str), 1163 (C=S), 1615 (C=N); ^{1}HNMR=(300MHz,DMSO)δ=1.10(3H,s), 2.1(2H,s), 2.23(2H,s), 3.75(3H,s), 4.15(1H,s), 6.42(1H,d), 6.55(1H,dd), 6.60(1H,dd), 6.66(1H,d), 7.5(2H,t), 8.0(2H,t)ppm, ^{13}C NMR: δ 186.0, 164.7, 158.8, 156.7, 152.4, 137.8, 126.3, 125.8, 122.3, 121.6, 115.5, 56.6, 30.5, 16.6; MS(EI) m/z=417.09[M]$^+$, Anal. calcd. for $C_{18}H_{19}N_5O_3S_2$: C,54.74; H,4.98; N,15.96; Found: C, 54.43; H, 4.64; N, 15.77.

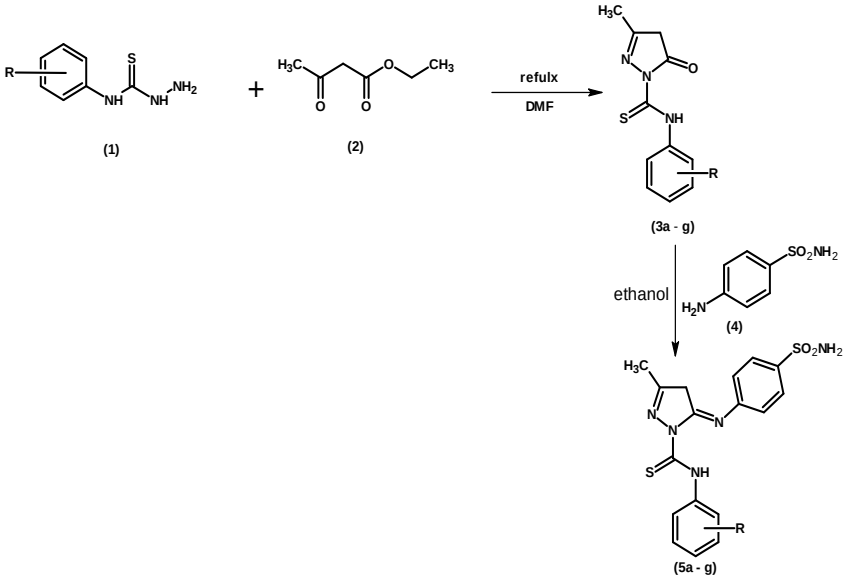
(5*E*)-*N*-(3-methoxyphenyl)-3-methyl-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5f)

Yield-68%, M.P.-145^{o}C, IR(KBr, ν_{max} cm^{-1}): 3245 (N-H-Str), 1660 (C=O), 1455 (C-N-Str), 1145 (C=S), 1612 (C=N); ^{1}HNMR=(300MHz,DMSO)δ=1.12(3H,s), 2.1(2H,s), 2.33(2H,s), 3.85(3H,s), 4.35(1H,s), 6.02(1H,s), 6.10(1H,d), 6.22(1H,d), 6.97(1H,dd), 7.5(2H,t), 8.0(2H,t)ppm, ^{13}C NMR: δ 186.7, 164.7, 162.3, 156.3, 152.2, 141.6, 137.8, 130.8, 126.8, 122.3, 118.6, 112.1, 110.9, 56.8, 30.8, 16.4; MS(EI) m/z=417.09[M]$^+$, Anal. calcd. for $C_{18}H_{19}N_5O_3S_2$: C, 55.84; H, 5.18; N, 16.36; Found: C, 54.93; H, 5.04; N, 16.17.

(5*E*)-*N*-(4-methoxyphenyl)-3-methyl-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5g)

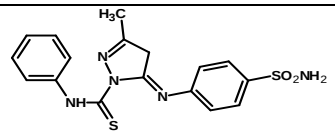
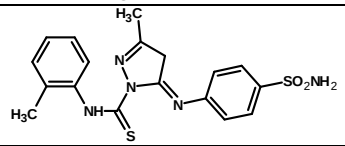
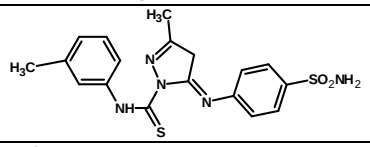
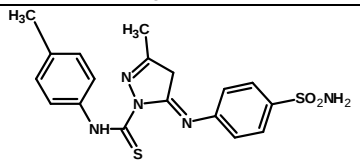
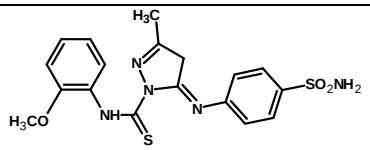
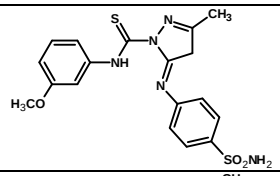
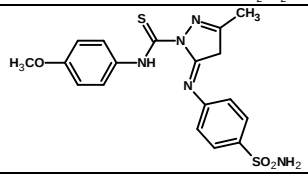
Yield-70% , M.P.-162^{o}C, IR(KBr, ν_{max} cm^{-1}): 3223 (N-H-Str), 1630 (C=O), 1435 (C-N-Str), 1159 (C=S), 1606 (C=N); ^{1}HNMR=(300MHz,DMSO)δ=1.05(3H,s), 2.1(2H,s), 2.30(2H,s), 3.81(3H,s), 4.32(1H,s), 6.44(2H,t), 6.59(2H,t), 7.5(2Ht), 8.0(2H,t)ppm, ^{13}C NMR: δ 186.2, 164.7, 158.0, 156.1, 152.1, 137.8, 132.2, 127.3, 126.3, 122.3, 115.4, 57.0, 30.2, 16.2; MS(EI) m/z=417.09[M]$^+$, Anal. calcd. for $C_{18}H_{19}N_5O_3S_2$: C, 54.64; H, 5.98; N, 15.99; Found: C, 54.43; H, 5.64; N, 15.87.

2.6 Synthetic Pathway:



Scheme – 1: Synthesis of pyrazolone Schiff base derivatives [R = H, CH_3, OCH_3 (o, m, p)]

Table 1: Physical characterization data of compounds 5a-g

S. No	Comp.	Strusture	Yield %	M.P. ^{o}C	Molecular formula	Colour
1	5a		68	120	$C_{17}H_{17}N_5O_2S_2$	cream
2	5b		70	215	$C_{18}H_{19}N_5O_2S_2$	Pinkish white
3	5c		67	159	$C_{18}H_{19}N_5O_2S_2$	white
4	5d		71	180	$C_{18}H_{19}N_5O_2S_2$	cream
5	5e		65	155	$C_{18}H_{19}N_5O_3S_2$	Creamish white
6	5f		68	145	$C_{18}H_{19}N_5O_3S_2$	Cream
7	5g		70	162	$C_{18}H_{19}N_5O_3S_2$	Creamish white

2.7 Antimicrobial studies

All the compounds were screened for their *in vitro* antimicrobial activity at the Birla institute of Medical Research and College of Life Sciences, Gwalior, against 24h old cultures of bacterial and fungal pathogens. Antimicrobial activity was determined against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial strains and *Candida albicans*, *Aspergillus niger* fungal strains using the disc diffusion assay. The zone of inhibition, observed around the disc after incubation was measured. The results are presented in **Table-2**.

Table 2: Antimicrobial activity data in MIC (µg/mL) of the compounds 5a-g

S. No.	Compound	Microbial strain			
		S. aureus	*P.aeruginosa*	*C.albicanes*	*A. niger*
1	5a	18	15	16	15
2	5b	15	17	14	17
3	5c	15	15	16	17
4	5d	15	16	13	14
5	5e	16	14	15	16
6	5f	15	17	17	18
7	5g	18	19	14	13
Chloramphenicol		24	24	-	-
Fluconazole		-	-	24	26

3. RESULTS AND DISCUSSION

The targeted pyrazolone Schiff base derivatives (**5a-g**) were obtained in excellent yields by refluxing substituted pyrazolones with sulfonamide in ethanol for 8 h. The reaction pathway has been summarized in **Scheme-1**. Newly synthesized compounds (**5a-g**) were characterized by IR, NMR, Mass and C, H, N elemental analysis.

Formation of (5*E*)-3-methyl-*N*-phenyl-5-[(4-sulfamoylphenyl)imino]-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (**5a-g**) were confirmed by recording their IR and ^{1}H NMR and ^{13}C NMR spectra. The IR (KBr) spectra of the target compounds **5a–g** showed characteristic N–H, C=O, C-N and C=S absorptions at 3,200–3,430 cm^{-1}, 1,600–1,750 cm^{-1}, 1400-1490 cm^{-1} and 1,150 cm^{-1} respectively. The ^{1}H-NMR ($CDCl_3$) spectra of these compounds exhibited the expected multiplet near δ 7.01-7.05 ppm due to the presence of aromatic protons. The N-H protons appeared singlets at δ 4.07–4.58 ppm respectively, whereas the pyrazolone methyl protons showed up near δ 2.50 ppm as a singlet. ^{13}C NMR spectra recorded signals corresponding to C=N, thiosemicarbazide moiety and other aromatic corbons. The mass spectrum of **5a** showed molecular ion peak at m/z = 387.08$(M)^{+}$, which is in agreement with the molecular formula $C_{17}H_{17}N_5O_2S_2$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and characterization is provided in **Table-1.**

4. CONCLUTION

Novel pyrazolone Schiff base derivatives were synthesized in reasonably good yields. They were characterized by Mass, ^{1}H-NMR, ^{13}C NMR, IR studies and elemental analyses. All the newly synthesized compounds were screened for their antimicrobial activity by MIC method. Among the screened samples, **5a** and **5g** have showed excellent antibacterial activity against *Staphylococcus aureus* bacteria as compared to the standard drug Chloramphenicol. They also showed similar activity as that of standard drug against selected bacteria and fungi. Compounds **5b** and **5g** were shown to inhibit the growth of *Pseudomonas aeruginosa* bacteria respectively. Although, a definite structure activity relationship could not be established with the limited experimental data and available compounds, it appears that with the incorporation of HC=N and $–NNH_2SCN$ in the resulting products **5** might have a positive influence, enhancing the antimicrobial activity of the designed compounds.

The antifungal activities of compounds **5a–g** against *Candida albicans* and *Aspergillus niger* fungal strain. The results of the *in-vivo* bioassay against are given in **Table-2**. Fluconazole was used as a reference antifungal drug. Compounds **5a, 5c** and **5f** were shown to inhibit the growth of *Candida albicans*, respectively; compounds **5b, 5c** and **5f** exhibited good activities on *Aspergillus niger* respectively. On the other hand, the remaining compounds showed moderately good activity against all of the tested bacterial and fungal strains compared to standard drugs, Chloramphenicol and Fluconazole. From the obtained results, here it is clear that the pyrazolone Schiff base play a major role for antimicrobial activity due to the aromatic rings which are bonded to N-C=S and HC=N moiety.

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